GENETICS OF CELL PROLIFERATION

M. Wigler J. Field, D. Young, J. Colicelli, K. Ferguson, T. Michaeli, G. Bolger, Y. Wang, R. Ballester, E. Chang, J. Gerst, G. Heisermann, M. Kawamukai, A. Vojtek, I. Wieland, H.-P. Xu, C. Nicolette, G. Asouline, K. O'Neill, M. Riggs, L. Rodgers, J. Brodsky, J. Douglas

Our laboratory focuses on two general areas: signal transduction and growth control. Much of our work centers about the RAS proteins, small guanine-nucleotide-binding proteins, which reside on the inner surface of the plasma membrane, that have been highly conserved in evolution and play a critical role in mediating signals that control cellular growth and other cellular events. We study the RAS proteins in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and in mammalian cells. In addition to RAS, we also study the families of mammalian cAMP phosphodiesterases, which play important roles in modulating the response of cells to cAMP. We plan to continue our studies of *ROS*, an oncogene that is expressed specifically in glioblastomas. Finally, we are developing a method for genomic difference cloning, which should enable scientists to discover new genetic abnormalities in cancer cells and search more effectively for new pathogenic organisms.

RAS in *Saccharomyces cerevisiae*

J. Field, R. Ballester, E. Chang, K. Ferguson, C. Nicolette, A. Vojtek, J. Colicelli, J. Gerst, T. Michaeli, M. Riggs, L. Rodgers, H.-P. Xu

There are two RAS proteins in *S. cerevisiae*, encoded by the *RAS1* and *RAS2* genes. They are highly ho-

mologous to the mammalian *RAS* genes. These yeast proteins are essential for growth and control the activity of adenylyl cyclase, the product of the *CYR1* gene. Activation of *RAS2* by point mutation (e.g., $RAS2^{val19}$) leads to activation of the cAMP signaling pathway and a consequent cluster of cellular phenotypes, including loss of tolerance to heat shock and sensitivity to nitrogen starvation. We have previously described the cloning of many genes that function along the RAS pathway, including *CDC25*, which encodes a factor that probably functions to regulate RAS by controlling guanine nucleotide exchange; *CYR1; BCY1*, which encodes the regulatory subunit of the cAMP-dependent protein kinase (cAPK); *TPK1*, *TPK2*, and *TPK3*, which each encode catalytic components of the cAPK; and *PDE1* and *PDE2*, which encode the low- and high-affinity cAMP phosphodiesterases, respectively. Using these genes, we have demonstrated a powerful feedback inhibition that maintains this system in homeostasis. We have demonstrated that the mammalian RAS proteins have been sufficiently conserved in evolution that they can function in yeast and stimulate yeast adenylyl cyclase in in vitro biochemical reactions. We have further demonstrated that RAS proteins must bind guanosine triphosphate in order to stimulate adenylyl cyclase. Finally, we have demonstrated through genetic analysis that it is highly likely that RAS proteins have additional functions in yeast (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]).

Many of the most essential questions about RAS in yeast remain unsettled: How is RAS itself controlled? How does RAS control adenylyl cyclase? Are there intermediate proteins required for this function? What are the other functions of RAS in yeast? We have made progress on these questions.

RAS in *S. cerevisiae*: How Is It controlled?

R. Ballester, E. Chang, K. Ferguson,
C. Nicolette, A. Vojtek

We have previously shown that RAS can stimulate its yeast effector only when bound to GTP. Thus, RAS may be controlled by factors that influence nucleotide binding. Two genes have been discovered in *S. cerevisiae* that are likely to encode proteins involved in this type of control of RAS. The first is *CDC25*, which encodes a product that probably catalyzes guanine nucleotide exchange on RAS. The second is *IRA1*, which encodes a protein that probably catalyzes GTP hydrolysis by RAS (Tanaka et al., *Mol. Cell. Biol. 9:* 757 [1989]). *IRA1* has slight homology with a mammalian gene called *GAP* (Trahey et al., *Science 242:* 1697 [1988]). GAP has been shown to induce GTP hydrolysis by RAS (Trahey and McCormick, *Science 238:* 542 [1987]). It has been proposed by other investigators that GAP is the effector for RAS (Adari et al., *Science 240:* 518 [1988]). In contrast, genetic analysis strongly suggests that IRA1 protein is involved with the down regulation of RAS (Tanaka et al., *Mol. Cell. Biol. 9:* 757 [1989]), and more specifically with its feedback control (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]; Ballester et al., *Cell 59:* 681 [1989]; Tanaka et al., *Mol. Cell. Biol. 9:* 757 [1989]). We have found that mammalian GAP, when expressed in yeast, does indeed down-regulate RAS (Ballester et al., *Cell 59:* 681 [1989]). It down-regulates both wild-type RAS2 and mammalian Ha-*ras*, when expressed in yeast, but not the activated $RAS2^{val19}$ protein, and can genetically complement $ira1^-$ yeast. These results suggest to us that mammalian GAP may be involved in the feedback inhibition of RAS in mammalian cells.

cAMP levels in yeast are regulated by glucose (Thevelein and Beullens, *J. Gen. Microbiol. 131:* 3199 [1985]). Other nutrient effects may be mediated through the cAMP effector pathway. We do not know the chain of events by which these signaling events occur. Other gene products besides those of *CDC25* and *IRA1* may be involved. To explore this area, we have begun searching for genes that, when overexpressed in yeast, can suppress the loss of *IRA1* or can suppress the loss of *CDC25*. Several new candidate yeast genes have been identified, and they will be characterized by sequence, genetic, and biochemical analyses. To perform biochemical analysis, we must first find conditions under which some component can affect the nucleotide bound to RAS. We are in the process of purifying CDC25 protein from yeast to determine under what conditions it can catalyze nucleotide exchange. We have shown that dominant interfering mutants of RAS can block function of wild-type RAS (Powers et al., *Mol. Cell. Biol. 9:* 390 [1989]). Interference is suppressed by overexpression of CDC25. We therefore proposed that these interfering RAS mutants form stable complexes with CDC25. We are in the process of testing this hypothesis by biochemical studies.

Our focus on the control of RAS is CDC25. For

reasons stated below, we believe it is likely that CDC25 has a mammalian homolog. Our studies in yeast thus will help guide experiments in mammalian cells. The fundamental question, both in yeast and in mammals, is the nature of the ultimate initiating signal for the pathway. In yeast, this initiating signal may originate from within the cell, perhaps a consequence of the metabolic state of the cell. There may be a similar initiating signal in mammalian cells.

RAS in *S. cerevisiae*: Its Interactions with Adenylyl Cyclase

R. Ballester, J. Colicelli, K. Ferguson, J. Field, J. Gerst, T. Michaeli, M. Riggs, L. Rodgers, A. Vojtek

We showed several years ago that the major effects of RAS proteins in yeast were explainable by their stimulation of adenylyl cyclase and that RAS proteins do indeed stimulate adenylyl cyclase in vitro. It has remained an open question whether this interaction is direct or whether it requires the presence of intermediary proteins. To help resolve this question, we are using extensive genetic and biochemical approaches. *S. cerevisiae* adenylyl cyclase made in *Escherichia coli* is neither full length nor RAS-responsive (unpublished results). To purify the adenylyl cyclase complex from yeast, we developed a method of epitope fusion and utilized immunoaffinity chromatography. This epitope fusion method is widely applicable for studying stable protein complexes. The adenylyl cyclase purified by this method copurifies with a tightly associated subunit with an apparent molecular weight of 70K (Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). We call this protein CAP for cyclase-associated protein. We have raised polyclonal antisera to CAP and identified the cDNA encoding CAP (Field et al., *Cell* [1990] in press) by screening an *S. cerevisiae* cDNA expression library (a generous gift from J. Kuret, Structure Section) with this antisera.

We have found that *CAP* is identical to a gene we had previously isolated, called *SUPC*. *supC* was a mutation that suppressed the phenotypes of activated *RAS*. In fact, cells that have disruptions of *CAP* do not respond to activated $RAS2^{val19}$, and the adenylyl cyclase purified from such yeast does not respond to RAS proteins in vitro.

We are currently testing if the coexpression of CAP and adenylyl cyclase in *E. coli* is sufficient to generate a RAS-responsive complex. At present, we know that these two proteins do form a complex when coexpressed in *E. coli*, but we do not yet know if other factors are required to form a RAS responsive complex. We are continuing the search for such factors by both biochemical and genetic means.

CAP is itself an interesting protein. Preliminary data suggest that it is conserved in evolution (see section on *S. pombe*, below). Disruptions of *CAP* lead to a severe phenotype that, at present, can best be explained by a failure to manage amino acid metabolism. We are in the process of testing this hypothesis and are looking at the interaction of CAP with GCN4, a protein involved in general amino acid metabolism (Hinnebusch, *Mol. Cell. Biol. 5:* 9 [1985]). The primary structure of CAP indicates that it contains two domains, separable by a stretch of prolines. Work in progress suggests that each domain has a distinct function: The amino-terminal domain is required for RAS responsiveness and the carboxy-terminal domain is required for amino acid response. We will continue to explore the function of CAP in an attempt to understand its role in mediating RAS effects, its role in mediating other signal transduction pathways in yeast, and its role in the evolution of signal transduction pathways.

We have also investigated the domains of adenylyl cyclase that appear to be required for interactions with RAS by assaying the RAS responsiveness of mutant adenylyl cyclase molecules in vitro. Figuring prominently in this work is a domain of cyclase that we call the leucine-rich repeat. The leucine-rich repeat occurs in the middle third of the adenylyl cyclase molecule and is composed of about 25 units of a consensus motif 23 amino acids in length. This motif is punctuated by proline and asparagine and contains leucine or an aliphatic amino acid every two to three residues. Similarly organized repeats have now been noted in a number of mammalian and insect proteins that form stable complexes with other proteins (Field et al., *Science 247:* 464 [1990]). Our work has shown that an adenylyl cyclase molecule with an amino-terminal deletion within 100 amino acids of the leucine-rich repeat is still fully RAS-responsive (Colicelli et al., *Mol. Cell. Biol.* [1990] in press). Deletions within the remaining molecule destroy RAS responsiveness. Small inframe insertion mutations reveal a different picture. Such mutations are generally without effect, except within the leucine-rich repeat. These data indicate that large-scale spacing of the adenylyl cyclase molecule is critical to its ability to respond to RAS, and the struc-

tural requirements within the leucine-rich repeat are especially rigorous.

We have discovered a second approach to studying RAS/target interactions. Mutant forms of adenylyl cyclase can actually interfere with the function of RAS, as evidenced by their ability to restore heat-shock resistance to strains carrying the *RAS2*^{val19} mutation (Field et al., *Science 247:* 464 [1989]). This observation led us to map the minimum region of the adenylyl cyclase molecule capable of interfering with RAS to the leucine-rich repeat itself. These studies also led to the design of genetic screens for the mammalian effectors of RAS (see below).

RAS in *S. cerevisiae*: Its Other Functions

T. Michaeli, A. Vojtek, H.-P. Xu

Although most of the functions of RAS in *S. cerevisiae* can be explained by effects on the cAMP signaling pathway, not all of its functions can be so readily explained (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). First, *ras1*$^-$ *ras2*$^-$ spores are not viable, whereas *cyr1*$^-$ spores are. Second, cells that are *ras1*$^-$ *ras2*$^-$ and suppressed by high-copy kinase genes, such as *TPK1* and *SCH9*, are temperature sensitive, whereas cells that are *cyr1*$^-$ and suppressed by these kinase genes are not temperature sensitive.

There is a third effect of RAS that is not readily explained by its effects on adenylyl cyclase. We have found that high levels of expression of an Ha-*ras* protein that has lost its carboxy-terminal processing site (e.g., Ha-*ras*^{ter186}) can suppress the phenotype of the *RAS2*^{val19} gene in yeast (Michaeli et al., *EMBO J. 8:* 3039 [1989]). Our biochemical and genetic analyses suggest that these types of Ha-*ras* mutants do not interfere with the processing of *RAS2*^{val19} or its interaction with adenylyl cyclase. Moreover, the mutant Ha-*ras* proteins must be bound with GTP to interfere. Our results are most readily explained if the mutants of Ha-*ras* bind to a second RAS effector, effectively competing for its binding with membrane-bound RAS. We think that this function has been conserved in evolution, since similar interfering effects of defective Ha-*ras* proteins are seen in *Xenopus* oocytes (Gibbs et al., *Proc. Natl. Aad. Sci. 86:* 6630 [1989]). Several candidate yeast suppressor genes of these mutant Ha-*ras* genes have been found and are being analyzed. These studies may lead to the discovery of RAS effector pathways that are truly conserved in eukaryotes.

RAS in *S. pombe*

M. Kawamukai, M. Riggs, L. Rodgers, Y. Wang, H.-P. Xu, D. Young

We have initiated the study of RAS in *S. pombe*, a fission yeast that is very diverged from *S. cerevisiae*. At the nucleotide level, *S. pombe* appears as diverged from *S. cerevisiae* as it is from mammals. In this organism, there is a single known homolog of RAS, called *ras1* (Fukui and Kaziro, *EMBO J. 4:* 687 [1985]). *ras1*$^-$ cells are viable, round, sporulation-defective, and mating-deficient (Fukui et al., *Cell 44:* 329 [1986]). *ras1* does not appear to operate through adenylyl cyclase. These differences between *S. pombe* and *S. cerevisiae* make the study of *ras1* in *S. pombe* particularly attractive.

We have focused on the function of *ras1* and have utilized two approaches. First, we are in the process of analyzing the adenylyl cyclase system of *S. pombe*. Knowledge of this system will allow us to compare and contrast *S. pombe* and *S. cerevisiae* RAS-responsive systems. We will be able to test *S. cerevisiae* components in *S. pombe* to learn more about their function. In particular, we will be able to learn if these components are RAS-specific or adenylyl-cyclase-specific. Second, we are in the process of a genetic analysis of suppressors of *ras1*-deficient cells, in the expectation that some suppressors will encode proteins in the *ras1* effector pathway. Progress has been made in both of these approaches. We have identified the *S. pombe* gene encoding adenylyl cyclase (Young et al., *Proc. Natl. Acad. Sci. 86:* 7989 [1989]). This gene encodes a protein that is highly similar to *S. cerevisiae CYR1*, including the carboxy-terminal catalytic domain and the leucine-rich repeats. Amino-terminal to the repeats is complete divergence. Overexpression of *S. pombe CYR1* leads to sterility in that organism. We have begun to look for suppressors of this phenotype. It is our expectation that *S. pombe* contains another RAS-like protein that controls its adenylyl cyclase activity.

The availability of the *S. pombe* system will enable us to examine the functions of *S. cerevisiae* genes more clearly. For example, we have already found that antibodies to *S. cerevisiae* CAP can be used to precipitate *S. pombe* adenylyl cyclase activity. A homolog to CAP must exist in this organism, and

we have begun searching for it in *S. pombe* expression libraries. By isolating this homolog, we will learn about the evolution of the domains of CAP, and this knowledge will help us to search for CAP homologs in higher organisms.

Our genetic analysis of the RAS pathway in *S. pombe* has centered around the search for genes that, when overexpressed, can suppress the phenotype resulting from *ras1* deficiency. To do this, we have created *S. pombe* strains lacking *ras1* or containing dominant interfering mutants in *ras1*, of the type that we found in *S. cerevisiae* which block CDC25 function (Powers et al., *Mol. Cell. Biol. 9:* 390 [1989]). Such cells are defective in sporulation. One gene was isolated from a high-copy chromosomal library that could suppress the interfering *ras1*, and we called it *sir1*. The nucleotide sequence of *sir1* indicates that it encodes a serine/threonine kinase. Genetic analysis indicates that the *sir1* product acts downstream from Ras1 protein and perhaps upstream of the product of *byr1*, another gene encoding a protein kinase that is capable of suppressing *ras1* deficiency (Nadin-Davis and Nasim, *EMBO J. 7:* 985 [1988]). Thus, it is likely that the *sir1* kinase is closer to the root of the *S. pombe ras1* pathway than is the *byr1* kinase. We are testing if *sir1* copurifies as part of a *ras1* responsive complex. In yeast, Sir1 protein appears to be exclusively membrane bound. We have succeeded in expressing Sir1 protein in *E. coli*.

Mammalian RAS

R. Ballester, E. Chang, J. Colicelli, K. Ferguson, J. Field, C. Nicolette, M. Riggs, L. Rodgers, A. Vojtek, H.-P. Xu

The parallels between the mammalian and yeast RAS pathways are striking. First, mammalian RAS can function in a yeast host and can stimulate yeast adenylyl cyclase in vivo and in vitro. Second, yeast RAS can function in mammalian cells to induce transformation (DeFeo-Jones et al., *Science 228:* 179 [1985]). Third, mammalian GAP can complement yeast deficient in *IRA1*. Fourth, we and other investigators have shown that Ha-*ras* mutants that are dominant interfering in yeast are dominant interfering in mammalian cells (Powers et al., *Cell 36:* 607 [1984]; Feig and Cooper, *Mol. Cell. Biol. 8:* 3235 [1988]). Most recently, we have shown that the yeast *SCD25* gene, a homolog of *CDC25* (Boy-Marcotte et al., *Gene 77:* 21 [1989]), can transform animal cells. Despite these similarities, the targets of RAS action appear to be different in yeast and vertebrates.

We have applied a genetic approach to identifying mammalian components that interact with RAS. We constructed mammalian cDNA libraries in yeast expression vectors and have searched these libraries for genes that can suppress mutations in the RAS pathway in yeast (Colicelli et al., *Proc. Natl. Acad. Sci. 86:* 3599 [1989]). We have utilized three types of screens, using both *S. cerevisiae* and *S. pombe* as hosts.

The first cDNA screen has been for genes that can suppress the heat-shock phenotype of cells with the $RAS2^{val19}$ mutation. The rationale for this screen is that, like the defective adenylyl cyclase gene, the mammalian effector of *RAS* might well interfere with activated RAS. Several candidates have emerged from this screen. One set encodes cAMP phosphodiesterases (see below). The second set is under evaluation. Genes from the second set do not appear to encode cAMP phosphodiesterases and bear no homology with previously identified nucleotide sequences in the data banks. At least one member of this set encodes a product that interferes with activated *ras1* in *S. pombe*. We expect that genes from this set either encode true inhibitors of RAS function (e.g., by blocking nucleotide exchange) or, more likely, are effectors of RAS that are not functional in yeast hosts.

In the second cDNA screen, we have sought cDNA genes that can suppress the loss of *ras1* in *S. pombe*. Several mammalian cDNAs have been isolated this way, and they are still under investigation. Among the genes so isolated have been Ha-*ras*, *RAP1*, and *RAP1A*. The latter two genes are members of the *RAS* superfamily (Pizon et al., *Oncogene 3:* 201 [1988]). *RAP1* was also isolated as Ki-*rev*-1, a gene capable of suppressing Ki-*ras* function in mammalian cells (Kitayama et al., *Cell 56:* 77 [1989]). Our results demonstrate that *RAP* genes and *RAS* genes can share effectors.

The third cDNA screen is designed to search for genes that can suppress *S. cerevisiae* with temperature-sensitive alleles of *CDC25*. We hope that this screen uncovers mammalian homologs of *CDC25*.

The *ROS* Oncogene

C. Birchmeier, K. O'Neill, M. Riggs, S. Sharma

The *ROS* oncogene was first discovered as the transforming principle of the avian retrovirus UR-2 (Neck-

ameyer and Wang, *J. Virol. 53:* 879 [1985]). We first encountered it as *MCF3*, an oncogene arising by rearrangement following a DNA transfer experiment. Subsequent studies revealed ROS to be expressed in a high proportion of human glioblastomas, but in very few other kinds of tumors, and not at all in normal glial cells. In glioblastomas, the predominant mRNA is about 8.0 kb. We have cloned cDNA to this mRNA from SW1088 and have completed the nucleotide sequence (Birchmeier et al., *Cell 43:* 615 [1985]). We have also cloned a cDNA to a 4.0-kb *ROS* mRNA found in another cell line, U118 (Sharma et al., *Oncogene Res. 5:* 91 [1989]). *ROS* can encode a transmembrane tyrosine kinase that shares homology with the product of the *Drosophila melanogaster sevenless* gene, both in the intracellular kinase domain and in the extracellular domain. Studies of *ROS* have shown that in two out of two glioblastomas, it has undergone mutation, consistent with the idea that the aberrant expression of ROS contributes to the malignancy of tumors of glial origins. We plan to continue these studies, examining the expression of ROS in primary brain tumors, using antisera to a ROS protein that we have developed. It is our hope that ROS protein will prove to be a useful cell surface marker for the diagnosis and treatment of tumors of glial origin.

Mammalian cAMP Phosphodiesterase Genes

G. Bolger, J. Colicelli, T. Michaeli, M. Riggs, L. Rodgers

In the course of analysis of mammalian cDNAs that can suppress the effects of activated RAS in yeast, we discovered several cDNAs encoding high-affinity cAMP phosphodiesterases (Colicelli et al., *Proc. Natl. Acad. Sci. 86:* 3599 [1989]). These phosphodiesterases are of the class inhibited specifically by rolipram, and they comprise a family of enzymes similar in structure to the product of the *dunce* locus in *D. melanogaster* (Chen et al., *Proc. Natl. Acad. Sci. 83:* 9313 [1986]). We have begun identifying and enumerating the species of phosphodiesterases expressed in human brain. The number of such genes expressed is surprisingly high. We are pursuing this project in the expectation that the characterization of the phosphodiesterases may be of medical value and in the hope that an understanding of the pattern of gene expression may lead to insights into the organization and evolution of neuronal cell populations.

A New Method for Genomic Difference Cloning

I Wieland, G. Bolger, K. O'Neill, G. Asouline

One common and fundamental problem of molecular biology confronts us when two similar genomes differ and we desire to understand the difference. One simple form of this problem can occur when a genome becomes deleted for sequences present in another due to germ line mutation, as can happen in genetic disease, or due to somatic mutation, as can happen during the development of cancer. Differences can also be acquired by infection with a DNA-based pathogen. Methods for identifying and isolating sequences present in one DNA population that are absent or reduced in another are called "difference cloning." Methods for difference cloning of cDNA populations have been widely described. Only one method for the difference cloning of genomic DNA is reported in the literature. This method was first described by Lamar and Palmer (*Cell 37:* 171 [1984]), who used it to clone sequences from the Y chromosome. Kunkel and co-workers used a variation of this method to clone fragments of the Duchenne's muscular dystrophy locus (Kunkel et al., *Proc. Natl. Acad. Sci. 82:* 4778 [1985]), which becomes deleted in some afflicted individuals. We have developed a different method for genomic difference cloning that is potentially more powerful (Wieland et al., *Proc. Natl. Acad. Sci.* [1990] in press). Although our method is not yet sufficient to isolate and define the small differences in genomes that would make it enormously useful as a tool for the study of neoplasia or infectious disease of viral origin, improvements are under way that will bring our method into that range. In its present state, our method is useful for the analysis of some genetic diseases and infectious diseases of unknown origin.

In our procedure for genomic difference cloning, sequences present in one genomic DNA population ("tester") are isolated that are absent in another ("driver"). By subtractive hybridization, a large excess of driver is used to remove sequences common to a biotinylated tester, enriching the "target" sequences that are unique to the tester. After repeated subtractive hybridization cycles, tester is separated from driver by avidin/biotin affinity chromatogra-

phy, and single-stranded target is amplified by the polymerase chain reaction, rendering it double-stranded and clonable. We have successfully modeled two situations: the gain of sequences that result from infection with a pathogen and the loss of sequences that result from a large hemizygous deletion. We obtain 100–700-fold enrichment of target sequences. We are in the process of improving the method by exploiting the second-order kinetics of self annealing. We are hopeful that we can soon begin to identify very small sequence differences between chromosomes.

PUBLICATIONS

Ballester, R., T. Michaeli, K. Ferguson, H.-P. Xu, F. McCormick, and M. Wigler. 1989. Genetic analysis of mammalian GAP expressed in yeast. *Cell* **59:** 681–686.

Colicelli, J., C. Birchmeier, T. Michaeli, K. O'Neill, M. Riggs, and M. Wigler. 1989. Isolation and characterization of a mammalian gene encoding a high-affinity cAMP phosphodiesterase. *Proc. Natl. Acad. Sci.* **86:** 3599–3603.

Michaeli, T., J. Field, R. Ballester, K. O'Neill, and M. Wigler. 1989. Mutants of Ha-*ras* which interfere with *RAS* effector function in *S. cerevisiae*. *EMBO J.* **8:** 3039–3044.

Powers, S., K. O'Neill, and M. Wigler. 1989. Dominant yeast and mammalian *RAS* mutants that interfere with the *CDC25*-dependent activation of wild-type *RAS* in *S. cerevisiae*. *Mol. Cell. Biol.* **9:** 390–395.

Sharma, S., C. Birchmeier, J. Nikawa, K. O'Neill, L. Rodgers, and M. Wigler. 1989. Characterization of the *ros1*-gene products expressed in human glioblastoma cell lines. *Oncogene Res.* **5:** 91–100.

Young, D., M. Riggs, J. Field, A. Vojtek, D. Broek, and M. Wigler. 1989. The adenylyl cyclase gene from *Schizosaccharomyces pombe*. *Proc. Natl. Acad. Sci.* **86:** 7989–7993.

In Press, Submitted, and In Preparation

Birchmeier, C., K. O'Neill, M. Riggs, and M. Wigler. 1990. Characterization of *ROS1* cDNA from a human glioblastoma cell line. *Proc. Natl. Acad. Sci.* (in press).

Colicelli, J., J. Field, R. Ballester, N. Chester, D. Young, and M. Wigler. 1990. Mutational mapping of RAS-responsive domains of the *Saccharomyces cerevisiae* adenylyl cyclase. *Mol. Cell. Biol.* (in press).

Field, J., A. Vojtek, R. Ballester, G. Bolger, J. Colicelli, K. Ferguson, J. Gerst, T. Kataoka, T. Michaeli, S. Powers, M. Riggs, L. Rodgers, I. Wieland, B. Wheland, and M. Wigler. 1990. Cloning and characterization of *CAP*, the *Saccharomyces cerevisiae* gene encoding the 70 kDa adenylyl cyclase associated protein. *Cell* (in press).

Field, J., H.-P. Xu, T. Michaeli, R. Ballester, P. Sass, M. Wigler, and J. Colicelli. 1990. Mutations of the adenylyl cyclase gene that block *RAS* function in *Saccharomyces cerevisiae*. *Science* **247:** 464–467.

Wieland, I., G. Bolger, G. Asouline, and M. Wigler. 1990. A method for difference cloning: Gene amplification following subtractive hybridization. *Proc. Natl. Acad. Sci.* (in press).